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In re application of: **LIU, Yu *et al.***	Confirmation No. **5810**
Appl. No. **10/607,584**	Examiner: **Vathyam, Surekha**
Filed: **June 27, 2003**	Art Unit: **1753**
For: **Improved Methods and Compositions for Capillary Electrophoresis (CE)**	Atty. Docket: **0219.0017C**

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Declaration of Yu Liu, Ph.D. Pursuant to 37 C.F.R. §1.132

Sir:

I, **Yu Liu, Ph.D.**, hereby declare as follows:

1. I believe that I am an original and joint inventor of the subject matter which is claimed and for which a patent is sought on the above-described patent application, the specification of which was filed on June 27, 2003, as Application Serial No. 10/607,584 (the "Application").

2. I am a scientist employed by the assignee (Beckman Coulter, Inc.) of the Application, and am a practitioner in the field of capillary electrophoresis and electrophoretic separation technology.

3. I am familiar with the specification and presently amended claims of the Application and have reviewed the Official Action dated March 6, 2007 (the "Official Action"), issued with respect to the Application.

4. I am aware that in the Official Action, claims 1-9, 11-13, 16-19, 21-27, 29-31 and 34-36 have been rejected pursuant to 35 U.S.C. § 103(a) as

obvious to one of ordinary skill in light of U.S. Patent No. 5,370,777 (Guttman *et al.* '777). I respectfully disagree.

A. I believe that those of ordinary skill would have understood Guttman *et al.* '777 to have concerned only the use of ***coated*** capillary tubes, and thus to be fundamentally different from the presently claimed invention.

B. I believe that coated capillary tubes are tubes that have been pre-treated to contain an internal coating designed to lessen the undesirable phenomena of electroendoosmotic flow and analyte-wall interactions that would otherwise distort the electrophoretic separation of analytes. Such phenomena arise from ionic repulsion occurring between the electrophoretic medium and the internal capillary wall. Such repulsion reflects the fact that both the medium (due to the addition of detergents such as sodium dodecyl sulfate) and the capillary tube (due to the ionization of its silanol groups) are negatively charged, and thus do not bind well to one another (thereby permitting spaces or channels to form at the capillary tube surface).

C. I believe that Guttman *et al.* '777 teaches that these problems are to be addressed using capillary tubes having a permanently affixed ***coating*** on their internal surface (Guttman *et al.* '777 at column 6, lines 44 – 51). The provided coating is stated to be formed from a bifunctional reagent having:

   (1) at least one positively charged amine (for ionically binding the reagent to the negatively charged capillary surface; Guttman *et al.* '777 at column 17, lines 8 – 13); and

   (2) a functional moiety suitable for cross-linking the reagent to the gel; Guttman *et al.* '777 at column 8, lines 8 – 10)).

I believe that the bifunctional reagent of Guttman *et al.* '777 thus served to bind the hydrophilic medium to the capillary wall, and to thereby lessen undesirable electroendoosmotic flow and analyte-wall interactions.

D. In contrast, the present invention involves aqueous gel media that employs a non-crosslinked hydrophilic polymer; a tris(hydroxymethyl)aminomethane – borate buffer; a detergent; and an organic additive. The composition unexpectedly produces an electrophoretic separation medium that can be used in ***uncoated*** capillary tubes in capillary electrophoresis to provide a molecular sieve. Significantly, the invention permits capillary electrophoresis to be conducted using polymers that do ***not*** bind silica. Such an advance is possible because non-silica absorbing polymers and the employed tris-borate buffer form complexes that are able to bind to the internal capillary surface and suppress. It was found that the gel medium does not suppress endoosmotic flow if the same polymer is dissolved in another type of buffer with the same concentration.

E. I believe that it would not have been obvious to those of skill in the art as of the filing date of the present application to have employed the claimed compositions in an uncoated capillary tube in capillary electrophoresis, in part because it would have been thought that ionic repulsion between the aqueous gel media and the capillary wall would have caused spaces or channels to form at the capillary tube surface and that such spaces would have caused undesirable electroendoosmotic flow and analyte-wall interactions that would distort the electrophoretic separation of the analytes.

5. I am also aware that the Examiner has advised that Guttman *et al.* '777 discloses the inclusion of reducing reagents such as dithiothreitol and 2-

mercaptoethanol with the sample, and that the Examiner has suggested that such reducing reagents, by virtue of their small size, would diffuse into the gel material and thus serve to keep analytes in a reduced form. I respectfully disagree. Guttman *et al.* '777 teaches the use of a reducing reagent solely for the purpose of sample preparation; i.e., "***before*** introduction into the capillary column" (see column 18, lines 41-42, emphasis added). I respectfully submit that the Examiner's conclusion fails to appreciate two consequences of such use:

A. The fact that analyte molecules, by virtue of their size and charge will migrate in capillary electrophoresis and thus will separate away from the uncharged reducing reagents. As a consequence of this fact, such reducing reagents, even if provided by Guttman *et al.* '777, would have been incapable of functioning to help *keep* protein analytes in a reduced form, as is presently claimed, since the protein analytes and the reducing reagents would have been continuously migrating further and further apart. Even if charged reducing reagents were employed, such molecules would have migrated faster than the larger protein analytes and would thus also have separated from such analytes.

B. The fact that any capability of such reducing reagents to help ***keep*** protein analytes in a reduced form in the method of Guttman *et al.* '777 is dependent on their being maintained at a concentration sufficient to mediate such function. In light of the active and/or differential mobility of the analytes and reducing reagents, I believe that including the reducing reagent in the sample preparation would not have provided a means for ***maintaining*** the concentration of such reducing reagents at a level sufficient to ***keep*** protein analytes in a reduced form.

6. I am also aware that claims 10 and 28 have been rejected pursuant to 35 U.S.C. § 103(a) as obvious to one of ordinary skill in light of U.S. Patent No. 5,370,777 (Guttman *et al.* '777) in view of U.S. Patent No. 3,622,661 (King *et al.*). King *et al.* is cited as evidence that dextrans having a non-cross-linked structure composed of approximately 95% alpha-D-(1-6) linkages are "conventional" in ***toothpastes***, and that, as such, the production of an ***electrophoretic separation medium*** containing such dextrans would have been obvious. I respectfully disagree.

   A. I believe that those of skill would not have considered the components of toothpastes as relevant to the constituents of an electrophoretic separation medium. I believe that the problems of concern to propounders of toothpaste compositions are distinct from, and do not overlap with, the problems of concern to those developing electrophoretic separation media.

   B. I believe that moreover King *et al.* fails to motivate, teach, suggest or predict the use of dextran compositions having the molecular weight recited in the present claims, and such weight is not an inherent characteristic of a dextran.

   C. I believe that since King *et al.* has no connection or relevance to capillary electrophoresis, those of ordinary skill would not have found in this document any teaching, suggestion or prediction to employ the disclosed toothpaste component in : (A) an uncoated capillary tube and (B) in an aqueous gel medium capable of facilitating the electrophoretic separation of analytes via capillary electrophoresis using an uncoated capillary tube by comprising a molecular sieve, as presently claimed.

7. I am also aware that claims 14-15 and 32-33 have been rejected pursuant to 35 U.S.C. § 103(a) as obvious to one of ordinary skill in light of U.S. Patent No. 5,370,777 (Guttman *et al.* '777) in view of U.S. Patent No.

5,213,669 (Guttman '669). Guttman '669 is stated to teach an aqueous gel medium having an alcohol that is glycerol. I respectfully disagree. I believe that Guttman '669 fails to teach, suggest or predict the unexpectedly improved capillary electrophoretic separation medium being claimed (evidence of such unexpected benefit is provided in Example 5 of the Application). Specifically, I find no teaching, suggestion or prediction in Guttman '669 to use a tris borate buffer in an aqueous gel medium, and no teaching, suggestion or prediction in Guttman *et al.* '777 to use an uncoated capillary tube or media for use in an uncoated capillary tube. Accordingly, I believe that the combined teachings of these documents would not have taught, suggested, or predicted the use of a tris borate buffer in an aqueous gel medium for use in an uncoated capillary tube.

8. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Respectfully Submitted,

Date: **June 6, 2007**

/Yu Liu/
Yu Liu, Ph.D.